of the mammalian skin, wherein the mammalian skin is selected from the group consisting of a human and a mouse.

Remarks

This Amendment is responsive to the Final Office Action of July 31, 2002. Applicant respectfully submits that this Amendment should be entered because it, Applicant believes, places the pending claims in condition for immediate allowance or removes issues for appeal.

I. Claims

Claims 1-40 were pending in the application and these claims stood rejected.

Claims 1, 18, 32 and 40 have been amended to clearly define the invention. Support for the recitation "lasting beyond natural life span of differentiated epidermal cells" can be found in the specification, for example, at page 16 line 5 through page 17, line 10. Applicant respectfully submits that no new matter is added by these amendments.

II. Rejections Under 35 U.S.C. § 112 Second Paragraph

Claims 1-17 stood rejected under 35 U.S.C. § 112, second paragraph as indefinite. Specifically, the Examiner avers that "the RNA-DNA oligonucleotide no longer has any functional limitation which clearly defines the activity." Applicant respectfully disagrees with this assertion. The Claim 1, like the claim 18, requires that stable genetic modification is made to the selected gene and this modification result in phenotypic changes at selected locations of the human skin after delivering a composition comprising the RNA-DNA oligonucleotide. Given these functional limitations, one skilled in the art would understand the metes and bounds of the claim when read in light of the specification. Accordingly, withdrawal of this rejection is respectfully requested.

III. Rejections Under 35 U.S.C. §102

Claims 32-34 and 37-39 stood rejected under 35 U.S.C. §102(b) as allegedly being

anticipated by Alexeev et al., 1998, Nature Biotechnology 16:1343-1346. Applicant respectfully traverses this rejection.

In maintaining this rejection, the Examiner averred that there is no evidence that the albino mouse disclosed in Alexeev et al., would be any different if it were generated by altering the genome of a normal mouse by using the instantly claimed method. In response, Applicant points out that the one skilled in the art can distinguish the albino mouse from the claimed mouse model by test breeding . The albino mouse, as it is known in the genetic literature, carries cc genotype giving a pure white animal with pink eyes. When mated together, two albino mice always capable of germline transmission of the cc genotype and produce only white albino mice. The skin disorder in the claimed animal model is a result of mutations induced in skin cells (which are somatic cells, not germ cells) and the skin disorder results in the treated animal itself. See, for example, the specification at page 29, line 7 through page 30, line 9. These animal models are incapable of germline transmission of the mutated gene and the claim 32 has been amended to reflect this language. Accordingly, Alexeev does not anticipate the rejected claims and withdrawal of this rejection is respectfully requested.

IV. <u>Rejections Under 35 U.S.C. §103</u>

Claims 1-40 stood rejected as allegedly being obvious over Yoon et al., 1996, Proc. Natl. Acad. Sci., 93:2071-2076 and Alexeev et al., 1998, Nature Biotechnology 16:1343-1346, in view of Uttam et al., 1996, Proc. Natl. Acad. Sci, 93:9079-9084, Christiano et al., 1994, Proc. Natl. Acad. Sci, 91:3549-3553 and Cole-Strauss et al., 1996, Science, 273:1386-1389. Applicant respectfully traverses this rejection.

The Examiner maintains that the Yoon, Cole-Strauss and Alexeev references demonstrate the feasibility of RNA-DNA oligonucleotide mediated correction of gene mutations in *in vitro* cultured cells and hence the claimed invention is obvious because these references provide a basis for the use of the methodology for mutating skin genes *in vivo*. As pointed out by the Applicant, these references, at most, are invitations to try RNA-DNA oligonucleotide mediated modification of genes in intact skin but do not suggest how that goal might be accomplished. In other words, the cited references may pique the curiosity of one skilled in the

art such that further investigation might be done as a result, but the references themselves do not contain sufficient teachings of how to obtain the end result or that stable genetic modifications can be made to a selected gene which result in phenotypic changes at selected locations of an animal skin. The cited language in the Yoon reference (which reference teaches the modification of episomal genes *in vitro*) merely suggests the exploration of this new technology. The Yoon reference gave no indication of which parameters were critical in extending the *in vitro* studies to intact organs such as skin. Similarly, Cole-Strauss and Alexeev, the secondary references, do not give any direction as to what parameters are needed or not needed for successful *in vivo* modification of skin genes. Applicant points out again that "obvious to try" is improper consideration in adjudicating obviousness issue. *In re Dow Chemical Co.*_ 5 USPQ2d 1529 (Fed. Cir. 1988); *Hybritech Inc. v. Monoclonal Antibodies, Inc.,* 231 USPQ 81 (Fed. Cir. 1986).

Applicant is aware that "for obviousness under 35 U.S.C. §103, all that is required is a reasonable expectation of success." As the Applicant already pointed out that there is no reasonable expectation of success from the reports demonstrating modification of genes *in vitro* without establishing a correlation to an intact skin situation. In general, it is known in the art that the modification of episomal genes can be more easily achieved than chromosomal gene modifications in cells. In fact, this distinction was noted by the skilled artisan as late as 2001 (See, Igoucheva et al., Gene Therapy, 2001, 8:391-399 reporting targeted gene correction in cells *in vitro*, a copy of which is enclosed herewith). Further, for example, Alexeev's *in vitro* approach uses melan-c cells and does not involve the intact skin and its complexity. The specification teaches, for example at pages 16-17, that (i) melanocytes account for only a minor portion (1%) of the total population of cells present in mouse skin, (ii) hair pigmentation may require correction of many melanocytes per hair follicle to produce and deposit enough melanin in hair shaft and (iii) the gene correction of one or two melanocytes per hair follicle may not be detected as a phenotypic change. Such issues coupled with the absence of any guidance in the cited references for successful modification of skin genes *in vivo* using RDO approach would lead one to believe that there is no reasonable expectation of success.

Further, gene correction in melan-c cells of Alexeev is limited only to those cells because these are terminally differentiated cells. When these cells are lost, the gene modification and the

associated phenotypic change, if any, would also be lost. The cited art does not teach how to bring about genetic modifications in skin cells using chimeric RNA-DNA oligonucleotides such that phenotypic changes are seen over a period of time lasting several cycles of cells. In the present invention it is shown that permanent gene correction by RDO did last during the life span of not only the corrected melanocyte cells but also in the renewed melanocyte cells. Neither Yoon, nor Cole-Strauss or Alexeev provide guidance on how to achieve the gene correction in differentiated cells (e.g., melanocytes, keratinocytes or fibroblasts) *in vivo* beyond the life span of such corrected cells. In the absence of such guidance in the cited art, there is no reasonable expectation of success.

Accordingly, because of the "obvious to try" nature of the cited references and lack of actual teachings or suggestions therein insofar as they affect the presently claimed invention, the *prima facie* case of obviousness is not made.

As to the unexpectedly high frequency of gene modifications pointed out by the Applicant, the Examiner avers that " would each and every embodiment provide an unexpected result commensurate in scope with [the claims]". First, Applicant points out that the *prima facie* case of obviousness is not made, as discussed above, regardless of whether the unexpected results are commensurate in scope with the claims. Second, as conceded by the Examiner, the unexpected results are in commensurate with at least certain embodiments (see, for example, claim 40).

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection are respectfully requested.

V. Conclusion

Applicant believes that this amendment places the application in condition for immediate allowance. Reconsideration and the early issuance of a Notice of Allowance are earnestly requested.

Respectfully submitted,

Date: December 27, 2002

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Marked Up Version of Claims in Serial No. 09/473,872 in response to the Office Action of July 31, 2002

1. (Four Times Amended) A method of modifying a selected gene in cells of a human skin in vivo which comprises delivering to said cells at one or more locations of the human skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that stable genetic modifications are made to the selected gene which result in phenotypic changes <u>lasting beyond natural life span of differentiated epidermal cells</u> at said locations of the human skin.

18. (Twice Amended) A method of modifying a selected gene in cells of an animal skin in vivo which comprises delivering to said cells at one or more locations of the animal skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that the stable genetic modifications are made to the selected gene which result in phenotypic changes <u>lasting beyond natural life span of differentiated epidermal cells</u> at said locations of the animal skin, wherein the animal is a mouse.

32. (Thrice Amended) A non-human animal model having a skin disorder at one or more locations of its skin wherein the skin disorder is a result of a treatment at said locations with a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops targeted to a selected skin gene, said oligonucleotide thereby causing a mutation in the selected skin gene which mutation leads to the skin disorder, <u>in said animal model, and said animal model is incapable of germline transmission of the mutated gene,</u> wherein the skin disorder is an epidermal fragility disorder, a keratinization disorder or albinism disorder.

40. (Twice Amended) A method of correcting a mutation in a tyrosinase gene in cells of a mammalian skin in vivo which comprises delivering to said cells at one or more locations of

the mammalian skin an effective amount of a composition comprising a Tyr-A RNA-DNA oligonucleotide for causing stable genetic correction in the tyrosinase gene and a pharmaceutically acceptable carrier such that the correction results in restoration of tyrosinase enzyme activity lasting beyond natural life span of differentiated epidermal cells at said locations of the mammalian skin, wherein the mammalian skin is selected from the group consisting of a human and a mouse.

APPENDIX: Pending claims in application Serial No. 09/473,872 after the entry of the amendment filed on December 27, 2002 in response to the Office Action dated July 31, 2002:

1. A method of modifying a selected gene in cells of a human skin in vivo which comprises delivering to said cells at one or more locations of the human skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that stable genetic modifications are made to the selected gene which result in phenotypic changes lasting beyond natural life span of differentiated epidermal cells at said locations of the human skin.

2. The method of claim 1, wherein the stable genetic modification is in an epidermal fragility disorder gene selected from the group consisting of COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14 and PKP1.

3. The method of claim 1, wherein the stable genetic modification is in a keratinization disorder gene selected from the group consisting of KRT1, KRT10, KRT9, KRT16, LOR, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP and DSG1.

4. The method of claim 1, wherein the selected gene is tyrosinase, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, PPO, BPAG2, or DSG3 gene.

5. The method of claim 1, wherein the selected gene is tyrosinase gene.

6. The method of claim 1, wherein the selected gene is COL7A1 gene.

7. The method of claim 1, wherein the selected gene is KRT17 gene.

8. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is, RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first string has one mismatching deoxyribonucleotide in said contiguous deoxyribonucleotides that defines a site of modification in the selected gene.

9. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least 20 ribonucleotides; and

(b) a second string of deoxyribonucleotides having the same number of deoxyribonucleotides as in the first string of nucleotides, wherein the second string is fully complementary to the first string of nucleotides except that the second string has a deoxyribonucleotide that forms a mismatched base pair with the corresponding nucleotide in the first string, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the deoxyribonucleotide in the second string also forms a mismatched base pair with

the corresponding deoxyribonucleotide in the DNA strand of the selected gene which mismatched base pair defines a site of modification in the selected gene.

10. The method of claim 1, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first and second strings have one, two or four pairs of nucleotide insertions or deletions that defines a site of modification in the selected gene.

11. The method of claim 1, wherein the stable genetic modification is correction of a mutation.

12. The method of claim 11, wherein the mutation is a point mutation or a frame shift mutation.

13. The method of claim 1, wherein the stable genetic modification is generation of a mutation.

14. The method of claim 13, wherein the mutation is a point mutation or a frame shift mutation.

15. The method of claim 13, wherein the mutation is a dominant mutation.

16. The method of claim 1, wherein said phenotypic changes include the correction of a skin disorder.

17. The method of claim 1, wherein said phenotypic changes include the correction of albinism, an epidermal fragility disorder or a keratinization disorder.

18. A method of modifying a selected gene in cells of an animal skin in vivo which comprises delivering to said cells at one or more locations of the animal skin an effective amount of a composition comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops and a pharmaceutically acceptable carrier such that the stable genetic modifications are made to the selected gene which result in phenotypic changes lasting beyond natural life span of differentiated epidermal cells at said locations of the animal skin, wherein the animal is a mouse.

19. The method of claim 18, wherein the selected gene is tyrosinase, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, KRT2, KRT6, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, PPO, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, a cytokine BPAG2 or DSG3 gene.

20. The method of claim 18, wherein the selected gene is tyrosinase gene.

21. The method of claim 18, wherein the selected gene is COL7A1 gene.

22. The method of claim 18, wherein the selected gene is KRT17 gene.

23. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxyribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first string has one mismatching deoxyribonucleotide in said contiguous deoxyribonucleotides that defines a site of modification in the selected gene.

24. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least 20 ribonucleotides; and

(b) a second string of deoxyribonucleotides having the same number of deoxyribonucleotides as in the first string of nucleotides, wherein the second string is fully complementary to the first string of nucleotides except that the second string has a deoxyribonucleotide that forms a mismatched base pair with the corresponding nucleotide in the first string to make the genetic modifications in the selected gene, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the deoxyribonucleotide in the second string also forms a mismatched base pair with the corresponding deoxyribonucleotide in the DNA strand of the selected gene which mismatched base pair defines a site of modification in the selected gene.

25. The method of claim 18, wherein the chimeric RNA-DNA oligonucleotide comprises:

(a) a first string of nucleotides wherein the first string is made of at least four contiguous deoxyribonucleotides flanked on each side by at least nine ribonucleotides; and

(b) a second string of deoxynribonucleotides that is fully complementary to the first string of nucleotides, and

wherein the chimeric RNA-DNA oligonucleotide is RNase protected, and wherein the chimeric RNA-DNA oligonucleotide has in each of the first and second strings a set of contiguous nucleotides that are fully complementary to a segment of DNA of the selected gene except that the first and second strings have one, two or four pairs of nucleotide insertions or deletions that defines a site of modification in the selected gene.

26. The method of claim 18, wherein the stable genetic modification is correction of a mutation.

27. The method of claim 26, wherein the mutation is a point mutation or a frame shift mutation.

28. The method of claim 18, wherein the stable genetic modification is generation of a mutation.

29. The method of claim 28, wherein the mutation is a point mutation or a frame shift mutation.

30. The method of claim 28, wherein the mutation is a dominant mutation.

31. The method of claim 18, wherein said phenotypic changes include the correction of albinism, an epidermal fragility disorder or a keratinization disorder.

32. A non-human animal model having a skin disorder at one or more locations of its skin wherein the skin disorder is a result of a treatment at said locations with a composition

comprising a chimeric RNA-DNA oligonucleotide having a double hairpin structure with pyrimidine loops targeted to a selected skin gene, said oligonucleotide thereby causing a mutation in the selected skin gene which mutation leads to the skin disorder, in said animal model, and said animal model is incapable of germline transmission of the mutated gene, wherein the skin disorder is an epidermal fragility disorder, a keratinization disorder or albinism disorder.

33. The animal model of claim 32, wherein the selected skin gene is Tyr, COL7A1, LAMA3, LAMB3, LAMC2, COL17A1, ITGA6, ITGB4, PLEC1, KRT5, KRT14, PKP1, KRT1, KRT10, KRT9, KRT16, LOR, 1998, KRT2e, KRT6a, KRT 16, KRT 17, STS, TGM1, GJB2, GJB3, ATP2A2, DSP, DSG1, HR, hHB1, hHB6, PAX3, TYR, TYRP-1, OCA2, OA1, MITF, HPS, FECH, UROS, URO-D, PPO, XPA, XPB, XPC, XPD, XPG, PTC, STK11/LKB1, PTEN, PTEN, XPB, XPD, WHN, GLA, ATM, ENG, ALK-1, a cytokine BPAG2 or DSG3 gene.

34. The animal model of claim 33, wherein the selected gene is Tyr gene.

35. The animal model of claim 33, wherein the selected gene is COL7A1 gene.

36. The animal model of claim 33, wherein the selected gene is KRT17 gene.

37. The animal model of claim 32, wherein the skin disorder is due to generation of a mutation in the selected skin gene.

38. The animal model of claim 37, wherein the mutation is a point mutation or a frame shift mutation.

39. The animal model of claim 37, wherein the mutation is a dominant mutation.

40. A method of correcting a mutation in a tyrosinase gene in cells of a mammalian skin in vivo which comprises delivering to said cells at one or more locations of the mammalian skin an effective amount of a composition comprising a Tyr-A RNA-DNA oligonucleotide for causing stable genetic correction in the tyrosinase gene and a pharmaceutically acceptable carrier such that the correction results in restoration of tyrosinase enzyme activity lasting beyond natural life span of differentiated epidermal cells at said locations of the mammalian skin, wherein the mammalian skin is selected from the group consisting of a human and a mouse.